



The combined effects of salinity and excess boron on mineral ion relations in broccoli[☆]

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ARTICLE INFO

Article history:

Received 18 August 2009

Received in revised form 26 January 2010

Accepted 31 March 2010

Keywords:

Boron distribution

Boron toxicity

Ion mobility

Ion partitioning

Ion relations

Salinity

Water reuse

ABSTRACT

Two plant stress factors, salinity and high levels of boron, often co-occur in natural and agricultural environments. Many investigations have been conducted to document the influence of the combined stresses on crop growth and yield. Only limited information, however, is available concerning the combined effects of the two stresses on mineral ion uptake and partitioning to shoot organs and tissues. Data for this study were obtained from an experiment conducted in greenhouse sand cultures with two water types: (1) a chloride-dominated system, and (2) sulfate-dominated waters characteristic of those present in the San Joaquin Valley of California. Each saline composition treatment was tested at three salinity levels (2, 12, 18 dS m⁻¹) and three B concentrations (0.046, 1.11, 2.22 mM; 0.5, 12, 24 mg L⁻¹). The gradient in B distribution in individual leaves sampled midseason was steep, with less boron accumulating in petioles than in the blades. The highest B concentrations (>100 mmol B kg⁻¹ dry weight) were found in leaf margins of plants grown in the low salinity-high boron treatments. These leaves were cupped upward, an unusual visual symptom characteristic of B toxicity. At final harvest, concentrations of B, Ca, Mg, Na, K, and Cl were highest in the oldest leaves on the broccoli shoots, decreasing acropetally to the heads. Total-P, however, was highest in broccoli heads, decreasing in the older tissues. Leaf-B accumulation was more closely related to salinity rather than to the concentration of Cl in the irrigation waters. Analysis of variance indicated that substrate B had little effect on Cl accumulation in salt-stressed broccoli leaves regardless of water type. No clear cut relationships were evident that would explain the reciprocal effects of B and Cl on crop yields.

Published by Elsevier B.V.

1. Introduction

Boron toxicity in plants is generally expressed as significant reductions of growth, vigor and yield, along with tissue damage, and loss of crop quality. In certain species, however, yield reductions may occur without visible shoot injury symptoms. For example, marketable yields of the crucifers, broccoli (*Brassica oleracea*, italica group), cauliflower (*B. oleracea*, botrytis group) and radish (*Raphanus sativus*), were significantly reduced when irrigated with waters containing 1.85 mM B, and yet no leaf injury was apparent in any of the three vegetables (Francois, 1986). Conversely, growth and yield of other species may not be restricted by high levels of external B, while, at the same time, their leaves show characteristic symptoms of severe B toxicity. Based on a 2-year trial

with *Eucalyptus camaldulensis* in outdoor sand tanks, Shannon et al. (1997) found no differences in total fresh biomass of the trees irrigated with saline waters (EC_{iw} = 22 dS m⁻¹) due to external B levels as high as 2.3 mM. However, leaves on trees irrigated with low salinity water containing high levels of B showed severe injury. The damage appeared initially as red blotches on interveinal tissues, and then moved progressively to leaf margins which eventually became necrotic (Grattan et al., 1997; Poss et al., 1999). Similarly, Marcar et al. (1999) found that growth of salt-stressed *E. camaldulensis* was insensitive to high external B, but that leaves were highly susceptible to B damage.

Patterns of leaf injury due to B toxicity are correlated with leaf venation and appear first at the end of the veins. Parallel-veined leaves [e.g., cereals, grasses, garlic (*Allium cepa*), onion (*A. sativum*)], generally show necrosis in leaf tips where the veins terminate. A similar pattern is found in lanceolate leaves [e.g., stock (*Matthiola incana*), carnation (*Dianthus caryophyllus*)] where the principal vein terminates in the tip. Species with obovate leaves such as geranium (*Pelargonium domesticum*) and broccoli, have radially distributed veins which show the effects of B toxicity as an injured zone all around the margin. In leaves with a well-developed network of

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veins, and with many veins ending in areas between principal side veins [gerbera (*Gerbera jamesonii*), aster (*Callistephus chinensis*), most eucalyptus and citrus species], symptoms first develop as interveinal yellow or red spots. As injury progresses, chlorosis spreads to the margins (Kohl and Oertli, 1961).

Typically, B moves passively from the roots with the transpiration stream, accumulating first in mature leaves where it becomes fixed and its retranslocation restricted (Brown et al., 1999b). In other species, however, the youngest tissues commonly contain the highest B levels resulting in toxic symptoms such as, terminal twig dieback, necrotic leaf spots, abnormal leaf forms and texture, bark cracking (Bradford, 1966).

The distinctly different distributional patterns reported for B accumulation and toxicity symptoms are closely related to the mobility of the ion in the plant. It is well documented that B is readily translocated in those plant species which biosynthesize sugar alcohols, forming phloem-mobile B-polyol complexes (Brown and Shelp, 1997). In those species, B is concentrated in young developing tissue where injury is manifested as 'twig die back' and burning of younger leaves and meristematic tissue. In most plants, however, B mobility is confined to the xylem because these species do not produce significant amounts of polyols. The two classes of plant species exhibit widely different patterns of B concentration gradients within the shoot in response to excessive levels of substrate B. Higher B concentration in mature, transpiring leaves appears to be an indication of B immobility; higher B concentration in young meristematic tissues is an indication of B mobility (Brown and Shelp, 1997; Brown et al., 1999a,b).

Boron movement in cruciferous vegetables, broccoli (Shelp, 1988), cauliflower (Shelp and Shattuck, 1987a), rutabaga (*B. napus*) (Shelp and Shattuck, 1987b), radish (Shelp and Shattuck, 1987c), occurs primarily in the xylem, accumulating in the mature leaves. However, if younger tissues become B deficient, B may be translocated from the source leaves in the phloem stream to supply the B requirements of the developing sinks (Shelp et al., 1995).

The visual injury symptoms of B toxicity and B distribution within the plant species given above are observed when B is the sole stress factor. Inasmuch as excess B is often present in soils and waters of agricultural regions where salinity is also a problem, it is relevant to consider how the performance, ion uptake, and appearance of B-stressed plants may change by the imposition of salt stress. Extensive research efforts have been directed towards investigating the effects of the combined stress factors, salinity and excess boron, on yield and quality of tomato (*Lycopersicon esculentum* Mill. (Ben-Gal and Shani, 2002); *Zea mays* L. cv. amylacea (Bastías et al., 2004); melon (*Cucumis melo* L.) (Edelstein et al., 2005); date palm (*Phoenix dactylifera* L.) (Tripler et al., 2007); pepper (*Capsicum annuum* L.) (Yermiyahu et al., 2007). Yermiyahu et al. (2008) recently reevaluated published data sets from studies reporting the combined effects of salinity and excess substrate B on the yield of crops such as wheat (*Triticum aestivum* L.) and chickpea (*Cicer arietinum* L.). They concluded that the interaction of the two stresses appears to be antagonistic, i.e. the outcome of the two factors, applied together, is less than the sum of the effects of either B or salinity, applied separately.

The response of plants to salinity depends not only on the total ion concentration in the external solution, but also on the kinds of salts contributing to salinity (Curtin et al., 1993). Traditionally, the majority of salt tolerance evaluation studies conducted at many institutions, including this Laboratory as well as at the Salt Tolerance Testing Laboratory at Swift Current, SK, Canada (Dr. Harold Steppuhn, personal communication) have been designed to use irrigation waters containing Cl as the sole salinizing anion (generally applied as a mixture of NaCl and CaCl₂). Soil solutions and irrigation waters in regions such as the San Joaquin Valley (SJV) of California and the prairies of western Canadian provinces, however,

contain substantial levels of SO₄ and it is reasonable to believe that plant response would differ, depending on the predominant anion in the saline substrate (Curtin et al., 1993). Recently, numerous salt tolerance studies have been conducted with irrigation waters that are more realistic, i.e., those prepared to mimic the compositions of sulfate-dominated drainage waters in the SJV (Shannon et al., 2000). The influence of these waters on growth and yield has been quantified for a wide variety of horticultural and agronomic crops, including leafy vegetables (kale (*B. oleracea* L.), pac choi (*B. rapa* L.), spinach (*Spinacia oleracea* L.) (Shannon et al., 2000), forages (Grattan et al., 2004), cut flowers (Carter et al., 2005). However, crop productivity in the Valley is also impacted by the presence of high levels of B in the saline soils and waters. It is important, therefore to understand the extent that boron may limit the long-term success of SJV agriculture.

This study was conducted to determine the combined effects of salinity and excess B on ion relations in broccoli, an important crop grown extensively in the SJV. Data were obtained from a greenhouse experiment in which broccoli was irrigated with boron-containing saline waters differing in water type (chloride- vs. sulfate-dominated) (Smith et al., 2010). Results of that portion of the study revealed that head yield and shoot biomass were reduced by both B and salinity. Furthermore, salinity significantly ameliorated the detrimental effects of excess B on crop performance. Objectives of the current portion of the study were to investigate the combined effects of salinity and excess B on: (1) visible signs of shoot injury due to B toxicity, (2) B partitioning among leaf components, (3) concentration and distribution of plant mineral nutrients and specific ions to shoot organs and tissues.

2. Materials and methods

The study was conducted at the U.S. Salinity Laboratory, Riverside, CA. The experimental was a randomized complete block design with two irrigation water types differing in ion composition (chloride-dominated vs. sulfate-dominated waters), three salinity levels (electrical conductivities (EC)=2, 12, and 19 dS m⁻¹), three B concentrations (0.046, 1.11, 2.22 mM; 0.5, 12 and 24 mg L⁻¹), and three replications. Seeds of broccoli cultivar Seminis PX511018 were sown on 4 February 2003 in greenhouse sand tanks. Tanks were irrigated twice daily with complete nutrient solutions for sufficient duration to completely saturate the sand. After irrigations, the solutions drained to individual 765-L reservoirs located below the sand tanks for reuse in the next irrigation. In addition to the macronutrients shown in Table 1 for the 2 dS m⁻¹ treatment, the solutions contained sufficient nitrate, phosphate, and essential micronutrients.

Boron and saline treatments were imposed on 20 February 2003 when the first true leaves were evident on 75% of the seedlings. Ion compositions of the saline treatments are shown in Table 1. The sulfate-dominated waters were prepared to simulate saline effluents commonly present in the shallow, saline water tables of

Table 1

Ionic compositions of waters for the various salinity treatments. SJV refers to San Joaquin Valley or sulfate-dominated waters and Cl refers to chloride-dominated water.

Salt type	ECw (dS m ⁻¹)	mM					
		Ca	Mg	Na	K	Cl	SO ₄
SJV	2	2.6	1.5	4.3	3.0	2.3	3.0
SJV	11	12.8	9.7	76.8	3.0	37.4	39.8
SJV	19	14.8	16.3	134.3	3.0	65.5	63.5
Cl	2	2.6	1.5	6.0	3.0	11.0	0.5
Cl	11	23.8	3.0	48.5	3.0	102.2	1.0
Cl	19	40.6	3.0	85.4	3.0	170.0	1.0

western San Joaquin Valley of California and from predictions based on appropriate simulations of what the long-term compositions would be upon further concentrations by plant water extraction and evapotranspiration (Suarez and Simunek (1997).

During the first few weeks of the study, the pH of both types of irrigation waters was adjusted to 6.0 with sulfuric acid every 2 days. We found that solution pH rose to about 8.0 over a 2-day period. Between 28 February and 15 April 2003, solution pH was adjusted to 6.0 approximately every 2 weeks. Thereafter, solution pH was not adjusted and was about 8.0 for the duration of the experiment.

Seedlings were harvested prior to bolting on 24 April 2003, 77 days after planting, 61 days under treatment. Intact blades, petioles, and blade margins (~3 cm wide) were sampled, washed in deionized water, oven-dried, ground to pass a 20-mesh screen, and stored for mineral ion analysis.

Final harvest took place on 21 May 2003. Shoots were divided into heads, stems, and leaves from the top, middle and lower sections of the shoot. Tissue samples were washed, dried and prepared for ion analysis. Sodium, K, Ca, Mg, total-S, and total-P were determined on nitric-perchloric acid digests of plant tissues by inductively coupled plasma optical emission spectrometry (ICPOES). Plant tissues were dry-ashed and B determined by ICPOES. Chloride was determined on nitric-acetic acid extracts by the coulometric–amperometric titration procedure.

Statistical analyses of ion data were performed by analysis of variance with mean comparisons at the 95% level based on Tukey's studentized range test. SAS release version 6.12 was used (SAS Institute, Inc., 2001).

3. Results and discussion

3.1. Injury symptoms of boron toxicity

With only one striking exception, the broccoli plants remained healthy and vigorous throughout the duration of the study, with no visible signs of shoot abnormalities due to ion toxicities or deficiencies (Fig. 1). That exception, a structural change observed on leaves of plants irrigated with solutions low in salinity (2 dS m^{-1}) but high in boron (2.22 mM), was present regardless of the dominant anion in the waters (Fig. 2). The disorder was characterized by slight bleaching of the marginal tissue followed by upward cupping of leaves. Cupping appeared on the first true leaf and, subsequently, on newer leaves as they reached full expansion. Leaf cupping has occasionally been reported as a symptom of B toxicity (Nable et



Fig. 1. Typical healthy broccoli seedlings grown in greenhouse sand tanks irrigated with saline waters ($\text{EC} = 19 \text{ dS m}^{-1}$) containing 2.22 mM B .



Fig. 2. Cupped broccoli leaves of seedlings irrigated with non-saline waters (2 dS m^{-1}) containing 2.22 mM B .

al., 1997). The direction of the cupping can vary, e.g. inwards, tomato (Alpaslan and Gunes, 2001); upwards, tomato (Ben-Gal, 2007); downwards, mint (*Mentha piperita* L.) (Loomis and Durst, 1992); or both upwards and downwards, celery (*Apium graveolens* L.) (Francois, 1988). Loomis and Durst (1992) suggested that the disorder may be due to inhibition of cell expansion, possibly by abnormal excess of cell wall cross-linkages.

3.2. Boron distribution in salt-stressed broccoli leaves

Boron distribution in leaves of broccoli seedlings followed the typical non-uniform pattern for plants with obovate leaves, i.e. increasing from petioles to the blade, and radially to blade margins (Fig. 3a and b). Under the low B treatment, the margins contained approximately twice as much B as the leaves and approximately 4-fold more B than the petioles. The effect of increased salinity on B concentration in the leaf components was not significant at low external B, averaging 8.2 , 5.3 , and 2.7 mmol kg^{-1} in the margins, blades, and petioles, respectively. As substrate-B increased, tissue-B increased significantly in all leaf components, and the effect of salinity was pronounced. The average boron concentration in intact broccoli blades from the low chloride salinity-high boron treatment was 39 mmol kg^{-1} , decreasing to 18 mmol kg^{-1} as salinity increased to 19 dS m^{-1} . The low salt-high B treatments also produced leaves with “cupped” margins containing 112 – 114 mmol kg^{-1} regardless of water type.

In an earlier study, broccoli cultivar ‘Waltham 29’ was irrigated with non-saline waters containing six B treatments ranging from 0.092 to 1.85 mM (Francois, 1986). Average B in entire blades was 87 mmol kg^{-1} , more than twice as high as in the Seminis variety used in the present study. The ratio of $B_{\text{margins}}:B_{\text{blades}}$ found in ‘Seminis’ broccoli was 3:1. If this relationship holds true for ‘Waltham 29’, then B content in ‘Waltham 29’ margins may have been in excess of 250 mmol kg^{-1} . Francois (1986) stated that he observed no visible toxicity symptoms or structural disorders associated with high leaf-B, providing yet another example of the lack of correlation between tissue-B concentration and leaf injury due to boron toxicity even in the same species (Wimmer et al., 2002). This observation also emphasizes that only limited information is available concerning differences in boron sensitivity among broccoli cultivars.

3.3. Ion distribution in broccoli shoot organs

A typical phloem-immobile element has been defined as one whose concentration increases from young to old leaves, whereas

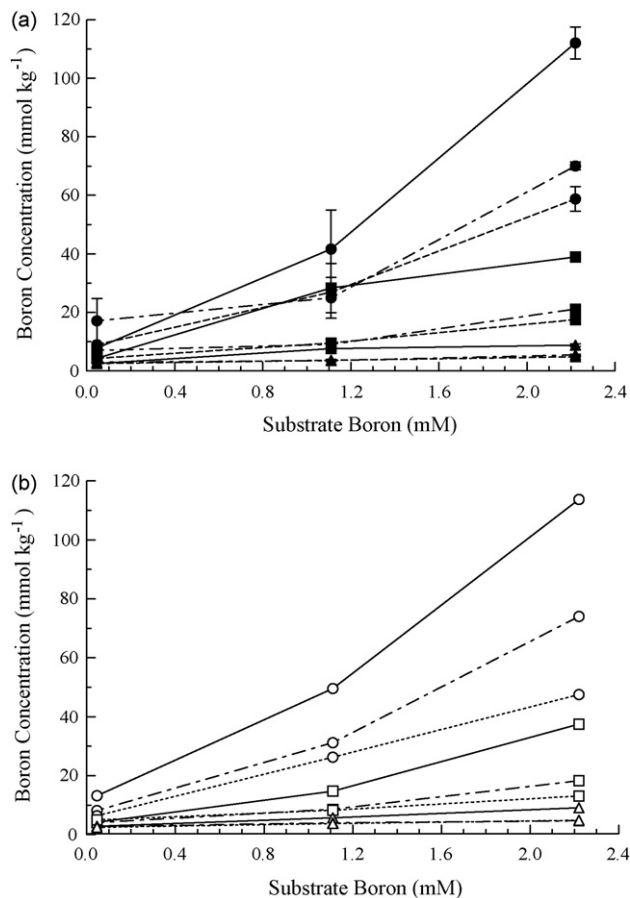


Fig. 3. (a) Boron distribution in broccoli petioles (Δ), blades (\square), and margins (\circ). Plants irrigated with chloride-dominated saline waters ($EC=2$ (—), 11 (---), 19 (---) $dS\ m^{-1}$) containing B (0.046 , 1.11 , 2.22 mM). (b) Boron distribution in broccoli petioles (Δ), blades (\square), and margins (\circ). Plants irrigated with sulfate-dominated saline waters [$EC=2$ (—), 11 (---), 19 (---) $dS\ m^{-1}$] containing B (0.046 , 1.11 , 2.22 mM). Data points for petiole boron concentrations in plants irrigated with saline waters ($EC=11$ and $19\ dS\ m^{-1}$) are superimposed.

the opposite is true for a typical phloem-mobile element (Van Goor and Van Lune, 1980; Brown et al., 1999a). That definition appears overly simplistic in view of the ion gradients found even under non-saline conditions in organs of different ages and at different positions along the broccoli axis in this study. Calcium, Mg, K, Na, Cl and B concentrations were all highest in the oldest leaves on lowest portion of shoot, decreasing progressively and significantly in younger leaves and the heads. The relative mobility of these ions has been well documented, with Ca and B rated as immobile ions; Mg, Na, K, P, S, and Cl as mobile ions (Marschner, 1995). However, mobility of some elements, such as B, may depend on the presence of sugar alcohols in certain plant species (Brown et al., 1999b).

In keeping with the definition of mobile and immobile ions cited above, Fig. 4 illustrates partitioning of an ion that is correctly rated as immobile in broccoli (B), one that is correctly rated as mobile (P), and one that is highly mobile (Na), but would be rated as immobile (Van Goor and Van Lune, 1980).

Boron distribution (Fig. 4A and D) was consistently highest in the oldest broccoli leaves and decreased in younger tissue, a finding that is in agreement with reports for many vegetable crops grown under non-limiting B supply (Oertli, 1993; Shelp, 1988; Shelp and Shattuck, 1987b). Under the low Cl-dominated salinity, B concentration decreased nearly an order of magnitude, from $80\ mmol\ kg^{-1}$ in the oldest leaves to $8\ mmol\ kg^{-1}$ in the heads. As salinity rose to 12 and $19\ dS\ m^{-1}$, B in mature leaves decreased

to 40 and $30\ mmol\ kg^{-1}$, respectively (Fig. 4A). Boron partitioning to shoot organs in response to irrigation with sulfate-dominated irrigation waters followed similar patterns (Fig. 4D).

Phosphorus, a highly mobile ion, is readily translocated from roots to the actively growing younger tissue, and is remobilized from senescent tissues to support reproductive development (Youngdahl, 1990). Many lower broccoli leaves were moderately chlorotic at final harvest, perhaps due to P-deficiency as P was transported to the developing heads. Total-P was lowest in the oldest broccoli leaves, and increased with increasing leaf age and height along the main axis. Heads formed on plants irrigated with the most saline chloride-dominated waters ($EC=19\ dS\ m^{-1}$; Fig. 4B) contained significantly higher levels of total-P than those irrigated with non-saline waters, whereas sulfate salinity had no effect on total-P in the heads (Fig. 4E).

Sodium is an example of a readily mobile cation that would, according to definition given above, be rated a non-mobile. A common strategy for plant adaptation to salinity is exclusion of potentially toxic ions (e.g., Na, Cl) from actively photosynthesizing tissues (Munns and Tester, 2008). Sodium exclusion mechanisms depend on the mobility of the cation and include: (1) compartmentation to older, mature organs (Yeo, 1983), and (2) retranslocation basipetally in the phloem to the roots where it may be confined to the basal zone or may be excreted to the external medium (Lessani and Marschner, 1978). Sodium in mature broccoli leaves was higher than in the heads (Fig. 4C and F). Sodium concentrations in shoot components from plants irrigated with sulfate-dominated waters were higher than in the chloride waters, reflecting the differential in substrate–Na concentrations between the two types of irrigation water (Table 1).

3.4. Effects of salinity and excess B on mineral ion relations

The youngest fully expanded leaves in the upper third of the broccoli shoot axes sampled at final harvest were considered reliable organs for characterizing the nutritional status of the whole plant as well as for identifying ion interactions, imbalances, or other relationships that may have affected growth and yield. Analyses of variances among Ca, Na, K, Cl and B content in these leaves sampled at the end of the experiment revealed significant differences due to the type of water used for irrigation. Boron treatment significantly affected leaf-Ca content, but had no effect on the other leaf-ions of plants irrigated with chloride-dominated waters. Conversely, salinity treatment had no effect on leaf-Ca, but significantly influenced leaf-Mg, -Na, -Cl, and -B (Table 2). In response to sulfate salinity, B treatment significantly affected Mg, Cl and B accumulation, whereas salinity treatment significantly influenced Ca, Mg, Na, K, Cl, and B concentrations in the leaves. However, the interrelationship between B and sulfate salinity treatments was significant only for leaf-B (Table 3).

Table 2

P values for the two-way ANOVA conducted to determine the interactive effects of excess boron and chloride-dominated irrigation waters on ion content in the youngest mature leaves of broccoli grown in greenhouse sand cultures.

Source	(mmol kg^{-1} dry weight)					
	Ca	Mg	Na	K	Cl	B
ANOVA						
Model	0.0761	<0.0001	<0.0001	0.0256	0.0002	<0.0001
r^2	0.4969	0.8200	0.8589	0.5704	0.7639	0.8751
P values						
B	0.0431	0.2609	0.4533	0.0953	0.1082	<0.0001
EC	0.2909	<0.0001	<0.0001	0.0301	<0.0001	0.0005
B \times EC	0.1542	0.0491	0.0062	0.0798	0.0507	0.0416

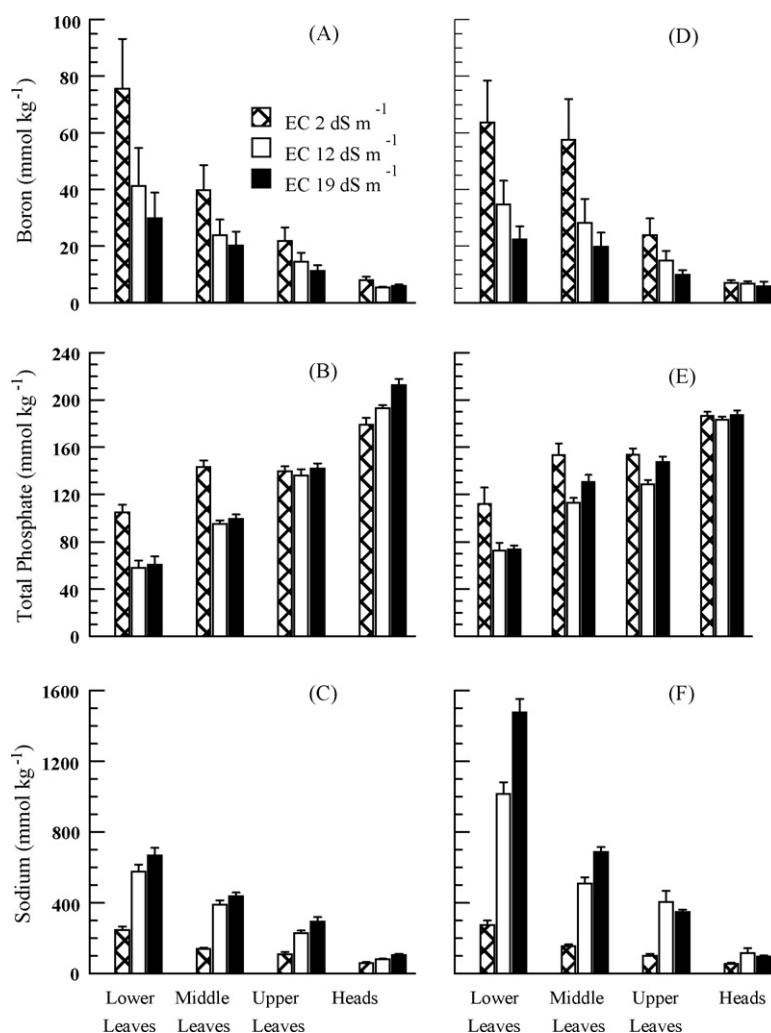


Fig. 4. Ion distribution in shoot components of broccoli irrigated with saline waters differing in ion composition and containing 0.046, 1.11, 2.22 mM B. Boron (A), sodium (B), total phosphate (C) in broccoli irrigated with chloride-dominated waters. Boron (D), sodium (E) and total phosphate (F) in broccoli irrigated with sulfate-dominated waters. Boron treatments are combined. Values are the means of three observations \pm S.E.

Regardless of water type, leaf-Cl was highest in the 11 dS m⁻¹ treatment and lowest in the 2 dS m⁻¹ treatment when substrate-B was low (Fig. 5). Leaf-Cl in plants irrigated with solutions low in chloride salinity decreased significantly and consistently from 450 to 215 mmol kg⁻¹ as external B increased from 0.046 to 2.22 mM (Fig. 5). In the 11 dS m⁻¹ treatment, leaf-Cl initially decreased from 830 to 500 mmol kg⁻¹ as external B increased from 0.046 to 1.11 mM, but then as solution-B increased, leaf-Cl increased 660 mmol kg⁻¹. Mean comparison of the data indicated that leaf-Cl was not statistically influenced by increases in substrate-B when chloride salinity was high (EC=19 dS m⁻¹), and averaged 640 mmol kg⁻¹ across the range of external B concentrations. Increasing substrate-B was correlated with decreases in leaf-Cl accumulation only at low and moderate chloride salinity, but not at high salinity. This finding is at variance with that of other investigators who report that for certain crops, Cl uptake and accumulation from chloride-dominated saline irrigation waters is negatively correlated with increases in substrate-B; i.e. tomato, *Lycopersicon esculentum* (Ben-Gal and Shani, 2002); pepper, *C. annuum* (Yermiyahu et al., 2008). In each of these studies, the irrigation waters were salinized with a single salt, NaCl, a complication which may explain the difference in results obtained in our experiment where more complex saline solutions were used.

Leaf-Cl tended to decrease in all sulfate salinity treatments as substrate-B increased. Mean comparison of the data from the 2, 11

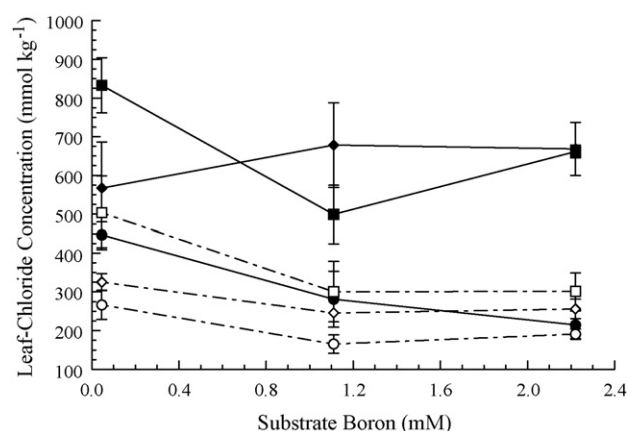


Fig. 5. Chloride concentrations in youngest expanded and expanding leaves of broccoli as a function of increasing substrate-B. Electrical conductivities in chloride-dominated irrigation waters were 2 (—●—), 12 (—■—), and 19 dS m⁻¹ (—◆—) and in sulfate-dominated irrigation waters were 2 (---○---), 12 (---□---), and 19 (---◇---) dS m⁻¹. Values are the means of three observations \pm S.E.

and 19 dS m⁻¹ treatments indicated that leaf-Cl was not statistically influenced by substrate-B and, across B treatments, averaged 210, 370 and 275 mmol Cl kg⁻¹, respectively (Fig. 5).

Analysis of variance indicated that, for both water types, leaf-B was significantly influenced by salinity ($P < 0.0005$), substrate-B ($P < 0.0001$) and the combined effects of salinity and boron ($P < 0.05$) (Table 3). The range of substrate-Cl concentrations was much wider for chloride-dominated waters than for the sulfate system (Table 1). Increases in external Cl in the Cl system had only a slight effect on leaf-B when substrate-B was low, but as substrate-Cl increased to 100 mM and solution B increased to 1.11 mM, leaf-B decreased from 25 to 11 mmol kg⁻¹, but thereafter, was not further affected as external Cl increased to 170 mM (Fig. 6). When external B was high, leaf-B declined significantly from 35 to 26 to 17 mmol kg⁻¹ as solution Cl increased from 11 to 102 to 170 mM.

Leaf-B in plants irrigated with low-B in the sulfate-dominated waters was also unaffected by increases in external Cl (Fig. 6). When external B increased to 1.11 mM, leaf-B decreased from 21 to 10 mmol kg⁻¹ as solution Cl increased, and at the highest B level (2.46 mM), leaf-B decreased from 46 to 15 mmol kg⁻¹ in response to increased substrate-Cl.

Leaf-Ca content was not correlated with increases in B in either the 2 or the 19 dS m⁻¹ chloride-dominated treatments, averaging 530 and 510 mmol kg⁻¹ across B levels, respectively (Fig. 7a). However, as B increased in moderately saline water (11 dS m⁻¹), leaf-Ca decreased from 700 to 530 mmol kg⁻¹, a response that was statistically significant ($P > 0.05$), but perhaps not biologically significant. The relative lack of increased Ca accumulation in broccoli leaves in spite of substantial increases in substrate Ca has also been observed in leaves of other cruciferous vegetables stressed with the same mixture of salts (Bernstein et al., 1974; Francois, 1994).

Across B treatments, leaf-Ca concentrations significantly decreased from 500 to 300 mmol kg⁻¹ as sulfate salinity increased to 19 dS m⁻¹, despite a five-fold increase in external Ca (Fig. 7a, Table 1). The complex nature of the SJV solutions leads to unusual ion ratios of Na/Ca, Mg/Ca, SO₄/Cl. High concentrations of substrate Na, for example, reduce Ca activity in the external media and limit plant-available Ca (Grattan and Grieve, 1999; Suarez and Grieve, 1988). Similar reductions in leaf-Ca with increasing sulfate-dominated salinity have been reported for other cruciferous vegetables irrigated with SJV waters (Grieve et al., 2001).

Leaf-Mg decreased from a mean of 120 to 80 mmol kg⁻¹ as Cl-salinity increased from 2 to 19 dS m⁻¹ (Fig. 7b) a response unaffected by increasing B levels. Reductions in leaf-Mg are often

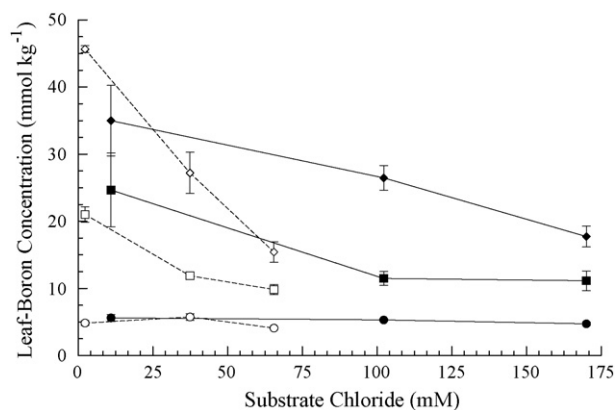


Fig. 6. Boron concentrations in youngest expanded and expanding leaves of broccoli as a function of substrate chloride in chloride-dominated irrigation waters (solid lines) and sulfate-dominated irrigation waters (dashed lines). Boron concentrations in irrigation waters = 0.046 (—○—), 1.11 (---□---) mM, and 2.22 (—◇—) mM. Values are the means of three observations \pm S.E. For some measurements, S.E. is smaller than the data point.

Table 3

P values for the two-way ANOVA conducted to determine the interactive effects of excess boron and sodium sulfate-dominated irrigation waters on ion content in the youngest mature leaves of broccoli grown in greenhouse sand cultures.

Source	(mmol kg ⁻¹ dry weight)					
	Ca	Mg	Na	K	Cl	B
ANOVA						
Model	0.0045	0.0079	0.0015	0.0173	0.0081	<0.0001
r ²	0.6600	0.6339	0.7054	0.5933	0.6325	0.9806
P values						
B	0.1728	0.0548	0.2222	0.3826	0.0094	<0.0001
EC	0.0002	0.0051	<0.0001	0.0012	0.0033	<0.0001
B \times EC	0.6606	0.0802	0.5482	0.3914	0.6101	<0.0001

associated with high concentrations of substrate-Ca. In this study, Mg in the chloride-dominated irrigation waters was slightly above nutrient requirement. Substrate Mg/Ca ratios were low, decreasing from 0.6 to 0.075 as salinity rose, and this ion imbalance likely account for the significant reduction in leaf-Mg.

In the sulfate salinity system, the effect of substrate-B on leaf-Mg was significant when salinity was low, i.e. in response to the 2 dS m⁻¹ treatment, leaf-Mg increased from 125 to 180 mmol kg⁻¹ as B increased from 0.046 to 2.22 mM (Fig. 7b). Leaf-Mg averaged 180 mmol kg⁻¹ at moderate and high salinity, regardless of substrate-B levels.

Sodium levels were slightly lower in shoots treated with chloride waters than sulfate-dominated solutions (Fig. 7c), a response that is directly related to differences in substrate-Na concentrations (Table 1). Across substrate B levels, leaf-Na increased from 150 to 295 mmol kg⁻¹ as chloride salinity increased from 2 to 19 dS m⁻¹, and from 100 to 350 mmol kg⁻¹ in response to increasing sulfate salinity. Regardless of irrigation water composition, boron treatment had little effect on leaf-Na accumulation.

Analysis of variance indicated that K concentrations in the youngest broccoli leaves were significantly ($P < 0.0001$) influenced by salinity, but not by B. Leaf-K contents were substantially higher than any other cation (Fig. 7d). Even under high salt stress, many cruciferous vegetables are very efficient K accumulators (Grieve et al., 2001). Broccoli proved to be no exception, and showed a strong preference for K over Na. Although the external K/Na ratio in 19 dS m⁻¹ sulfate irrigation waters was low (0.022), the K/Na ratio (2.5) in the diagnostic leaves was well above the K/Na ratio of 1 which is considered adequate for normal functioning of metabolic processes. Salinity-induced reductions in leaf-K were significant, but relatively small, e.g., as chloride salinity increased from 2 to 19 dS m⁻¹, leaf-K decreased from 1110 to 995 mmol kg⁻¹ and, with increases in sulfate salinity, decreased from 1060 to 850 mmol kg⁻¹. This result is consistent with that of other studies where increasing Na is correlated with reduced K uptake regardless of the major anion in the irrigation waters (Grattan and Grieve, 1999; Grieve et al., 2001).

Except for a slight, but significant, increase in total-P (from 130 to 155 mmol kg⁻¹) in response to chloride salinity at 11 dS m⁻¹ waters containing 1.11 mM B, total-P in broccoli leaves was not otherwise affected by the type of saline water used for irrigation. Total-P in the leaves averaged 140 mmol kg⁻¹ across both salinity and B levels (Fig. 7e).

SJV waters were richer in sulfate than the Cl-dominated waters (Table 1) and as a result total-S values were higher in plants irrigated with sulfate-based solutions (Fig. 7f). Total-S in leaves of plants irrigated with waters low in chloride salinity increased significantly (from 390 to 515 mmol kg⁻¹) as substrate-B increased from 0.046 to 2.22 mM. Increases in chloride salinity had no effect on total-S in broccoli leaves when substrate-B was low. Total-S concentrations averaged 385 mmol kg⁻¹ in the 12 and 19 dS m⁻¹

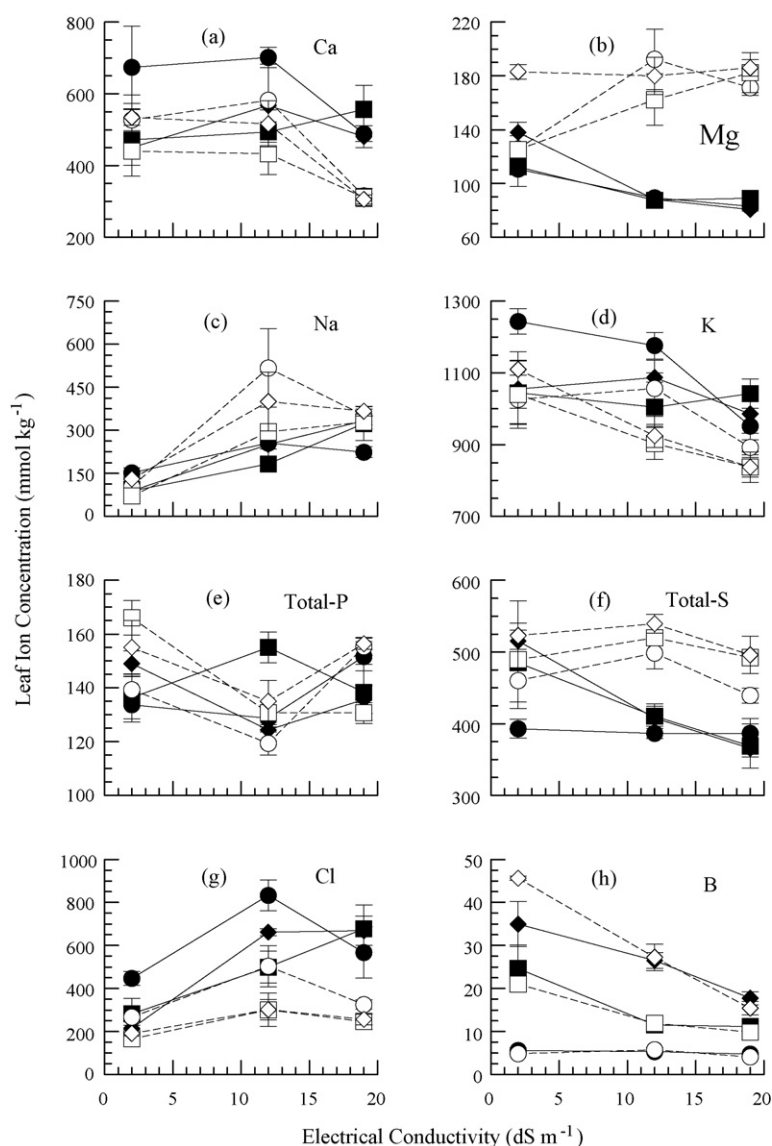


Fig. 7. Concentration of calcium (a), magnesium (b), sodium (c), potassium (d), total-P (e), total-S (f), chloride (g) and boron (h) in the youngest expanded and expanding leaves of broccoli as a function of increasing EC in chloride-dominated waters containing 0.046 (●-), 1.11 (-□-), and 2.22 (-◇-) mM B and in sulfate-dominated irrigation waters containing 0.046 (-○-), 1.11 (-□-), and 2.22 (-◇-) mM B. Values are the means of three observations \pm S.E. For some measurements, S.E. is smaller than the symbol.

treatments, regardless of substrate-B concentration. Mean comparison of the data indicated that total-S in leaves of plants irrigated with SJV waters was not influenced by either stressor and averaged 495 mmol kg^{-1} across solution B concentrations and salinity levels.

Substrate-B was negatively correlated with leaf-Cl accumulation at two levels of chloride salinity: leaf-Cl was reduced from 450 to 215 mmol kg^{-1} at low salinity as external B rose from 0.046 to 2.22 mM (Fig. 7g). Similarly, a reduction in leaf-Cl from 830 to 500 mmol kg^{-1} occurred when moderate chloride salinity (11 dS m^{-1}) was accompanied by an increase in external B from 0.046 to 1.11 mM. Chloride in leaves of plants irrigated with 19 dS m^{-1} waters, however, was not affected by B treatment, averaging $640 \text{ mmol Cl kg}^{-1}$ across B levels (Fig. 7g).

Mean comparison of the data indicated that large increases in both salinity and external Cl in the sulfate-dominated waters were not correlated with significant increases in leaf-Cl regardless of substrate-B concentration. Leaf-Cl averaged 210, 370, and 295 mmol kg^{-1} as EC of the irrigation water increased from 2 to 11 to 19 dS m^{-1} , respectively (Fig. 7g). Great care must be taken interpreting Cl relations in plants irrigated with SJV waters which

contain high concentrations of both SO_4 and Cl. Other vegetable crops, e.g., endive (*Cichorium endivia*), radicchio (*C. intybus*), and chard (*Beta vulgaris*) (Grieve et al., 2001), as well as the floral crop, *Limonium perezii*, (Carter et al., 2005) also irrigated with highly saline SJV waters showed a similar response. In both of these studies, 10- to 30-fold increases in external Cl did not significantly affect leaf-Cl accumulation in sodium sulfate-dominated waters. The increase in substrate-Cl, however, was accompanied by a 20-fold increase in substrate- SO_4 . These observations serve to illustrate that, for certain plants, the selectivity for SO_4 is greater than for Cl, and that substrate SO_4 may inhibit Cl uptake (Fixen, 1993). White and Broadley (2001) point out that anion channels in the plasma membrane facilitate Cl uptake. The channels, however, are also permeable to other anions (NO_3 , SO_4) which often limit Cl transport to the shoot.

Analysis of variance indicated that B accumulation in leaves of broccoli plants irrigated with chloride-dominated waters was significantly influenced by substrate B ($P < 0.0001$), chloride salinity ($P = 0.0005$) and the combined effects of salinity and B ($P < 0.05$). Leaf-B increased significantly at each salinity level as external B

increased, e.g. from 5 to 35 mmol kg⁻¹ in the 2 dS m⁻¹ treatment, and from 5 to 18 mmol kg⁻¹ in the 19 dS m⁻¹ treatment (Fig. 7h). As salinity increased and substrate B remained low, leaf-B was constant at 5 mmol kg⁻¹, a value that meets the B requirement for broccoli (Francois, 1986; Shelp, 1988). This finding is reassuring in that it indicates crop nutrient requirement for B will be adequate even at elevated salinity, regardless of the water type. As substrate B increased to 1.11 mM and chloride salinity increased from 2 to 11 dS m⁻¹, leaf-B decreased significantly from 25 to 11 mmol kg⁻¹. The effect of further salt stress, however, was not significant. Leaf-B decreased consistently and significantly from 35 to 27 to 18 mmol kg⁻¹ in plants irrigated with chloride waters containing 2.22 mM B.

Leaf-B in plants irrigated with sulfate-dominated waters was significantly influenced by substrate B ($P < 0.0001$), salinity ($P < 0.0001$), and by the interactive effects of both stressors ($P < 0.0001$). Leaf-B accumulation in plants irrigated with SJV waters was similar to that described above for broccoli grown in the chloride system (Fig. 7h). Other investigators also report that irrigation with SJV waters result in reduced B accumulation as well as alleviation of B toxicity symptoms in tall wheatgrass (*Thinopyrum ponticum*) (Diaz and Grattan, 2009); *E. camaldulensis* (Poss et al., 1999); several *Prunus* rootstocks (El-Motaïum et al., 1994).

Comparison of leaf-B patterns plotted against salinity (Fig. 7h) shows convergence of the lines compared to the same data plotted against Cl concentrations of the irrigation waters (Fig. 6) suggesting that, regardless of solution composition, B uptake depends more on EC than on solution Cl concentration. Values for leaf-B expressed in terms of EC were similar to those obtained when salinity was expressed as osmotic potential. (Data not shown.) Conversely, the influence of substrate-B on leaf-Cl was less clear. Reduction in Cl accumulation in diagnostic leaves was significantly correlated with increases in external B only at the lowest salinity level, regardless of irrigation water type. Other investigators find that Cl accumulation in salt-stressed plant tissues appears to be consistently and significantly reduced by substrate-B (Yermiyahu et al., 2008). One explanation for the differences may lie in the range of salinities and external B concentrations used, the composition of the irrigation waters, and/or the crop species tested in the studies. As an example: Yermiyahu et al. (2008) imposed four levels of NaCl-salinity (EC = 2.8, 3.9, 5.0 and 6.1 dS m⁻¹) and four substrate-B concentrations (0.046, 0.37, 0.74 and 1.1 mM) on the moderately salt sensitive pepper. In contrast, we tested the response of broccoli, a moderately salt-tolerant crop, to three levels of salinity containing NaCl and CaCl₂ (EC = 2, 12, and 19 dS m⁻¹), and three substrate-B concentrations (0.046, 1.11 and 2.22 mM). Thus, the high levels of salinity we used in the broccoli study, coupled with the very high concentrations of substrate-Cl, may have obscured the effects of B on Cl uptake and accumulation.

With few exceptions, external B appeared to exert little effect on accumulation of mineral ions in broccoli leaves. The results of this study reinforce the concept advanced by Yermiyahu et al. (2008): if the effect on one stress factor on plant response is particularly strong, the influence of the second stressor will be minor and is unlikely that statistical analysis of the data will produce useful results. Leaf-ion content in broccoli was primarily influenced by salinity. Certain unique responses in mineral ion accumulation in the salt-stressed plants depended on the ion composition of the irrigation water, and, specifically, whether the dominant anion in solution was sulfate or chloride.

Acknowledgements

The authors acknowledge the dedicated efforts of Phyllis Nash for statistical analyses and Donald A. Layfield for mineral ion analyses. John Draper and Doug Diaz provided technical assistance.

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